

Analysis of abiotic factors associated with foam nests of Cuvier's foam froglet (*Physalaemus cuvieri*) in southeastern Brazil

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Abstract

Climate change may have significant impacts on amphibian diversity due to alterations in microhabitat conditions where these species occurs. Understanding the abiotic factors associated with a species' microhabitat are therefore necessary to evaluate the impact they may suffer. Amphibians are exposed to changing microhabitat conditions at multiple life stages, since reproduction and larval development of most species depend on water, whereas adults frequently depend on terrestrial habitats. *Physalaemus cuvieri* is a Neotropical frog that uses foam nests for reproduction that may provide some protection for tadpoles against temperature and humidity fluctuations. Herein, foam nests of *P. cuvieri* were studied within vegetation around a pond, with the aim of analyzing the morphometric (depth, area and volume) relationships of foam nests with abiotic factors (humidity, temperature). Humidity 2 cm above the nests was significantly greater than 2 m from the nests. Temperature, measured at different depths of the nests, was significantly reduced by up to 10 °C when compared to atmospheric air temperatures above the nests. We conclude that foam nests facilitate a protective environment for eggs by regulating temperature and humidity to acceptable levels.

Keywords

Area/volume relation, humidity, morphometric measurements, temperature, thermal conduction

Introduction

Amphibian populations worldwide are declining. A growing concern among herpetologists is the influence of global warming on reproductive success, the quality of microhabitats, and interactions between amphibians and their pathogens (Costa et al. 2012). Studies that focus on abiotic factors associated with microhabitats occupied by amphibians have been shown to be important in predicting the possible deleterious effects of climate change (Hutchison and Dupré 1992; Rome et al. 1992; Ultsch et al. 1999; Wells 2007; Navas et al. 2008; Costa et al. 2012). Using local anuran populations as experimental models in the face of climate change in their microhabitats could contribute to broader predictions and to the implementation of more appropriate preservation and conservation measures for anurofauna (Toledo et al. 2010).

The family Leptodactylidae Werner, 1896, with 220 species currently described (Frost 2020), constitutes an interesting group as they present a diversity of reproductive modes (Haddad and Prado 2005), which according to Salthe and Duellman (1973), are defined as a combination of traits starting with types of spawning, oviposition sites, embryonic development rate, type of parental care, among others. In general, among anurans, twenty-nine reproductive modes are described by Duellman and Trueb (1986), and another seven reproductive modes were added by Haddad and Prado (2005) for amphibians in the Atlantic forest. Fourteen reproductive modes are recognized in Leptodactylidae, including the construction of foam nests as tendency towards greater terrestrially in reproduction in this family. *Physalaemus cuvieri* Fitzinger, 1826 belongs to the subfamily Leiuperinae Bonaparte, 1850 (Frost 2020) and could represent an important experimental model, since it possesses a wide latitudinal distribution throughout the Brazilian territory. *Physalaemus cuvieri* is found in the Northeast, Central, Southeast and South regions, as well as possibly in Venezuela, Argentina, and Paraguay (Frost 2020). It is a terrestrial nocturnal species found in open areas of several biomes such as Atlantic Forest, Cerrado and Pantanal, up to 2,000 m altitude (Haddad et al. 2008; Araujo et al. 2009). Males are commonly observed vocalizing among the vegetation on grasslands or in small clumps, mainly in flooded areas or on the margins of permanent or ephemeral water bodies. *Physalaemus cuvieri* has a lengthy reproductive season during the rainy season, from September to March (Bokermann 1962). Mating occurs in small depressions in the soil, where foam nests containing up to 600 eggs are being produced, connected to grass stems at the water's edge (Barreto and Andrade 1995; Gambale and Bastos 2014; Mijares et al. 2010). The species has adapted to anthropic environments and is currently not threatened within the Brazilian territory (IUCN 2020).

Foam nests, produced through limb motions beating oviduct secretions into foam during egg deposition, are a feature of the genus *Leptodactylus*, shared by certain other leptodactylid genera including *Physalaemus*, and by the family Rhaco-

phoridae, in South America and Australia (Heyer 1969). Foam nests could have several functions, such as protection against desiccation of eggs, embryos and larvae (Heyer 1969, 1975), protection against predators (Downie 1990b, 1993), adequate oxygen supply (Seymour and Loveridge 1994), growth inhibition (Pisano and Del Rio 1968), food source (Tanaka and Nishihira 1987), and maintaining adequate temperatures for development (Dobkin and Gettinger 1985). The present study aimed to better understand how the nests of *P. cuvieri* relate to abiotic factors, such as humidity and temperature, thus we aimed to determine: (1) if there are differences between the relative humidity of the air around the nests and above the nests; (2) whether there would be differences between the external (just above the nests) and internal nest temperature; and (3) how nest morphometry could be related to heat exchanges. These questions are important, since environmental issues related to global warming have been taken towards the conservation and preservation of amphibian populations worldwide, especially in the case of the species *P. cuvieri*, which has a wide distribution in Brazilian ecosystems.

Methods

Study area and data collection

The present study was carried out around a pond (20°21'44"S, 47°46'26"W; Fig. 1) located in the municipality of Ituverava, northeastern state of São Paulo, southeastern Brazil. According to Carrer and Garcia (2007) and following the Köppen–Geiger climate classification system, the climate of the municipality is considered to be of the AW tropical humid kind, characteristic of tropical savannas with humid summers and dry and mild winters, with temperatures of the coldest months above 18 °C.

Data were collected out on a single day in November 2012, between 15:00 and 18:00 hours, mapping 10 foam nests from *P. cuvieri* that were arranged on the banks of the lagoon, where males were seen vocalizing among the bushes (Fig. 1C). The present work was conducted under a Permanent License for the collection of zoological material, number 19125-2, provided by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA).

Distribution and morphometric measurements

After mapping the nests in the study area, data on morphometric measurements were collected, such as: (1) depth (D), considered as the central vertical axis between the opening and the bottom of the nests, measured with the aid of a caliper (Eccofer); (2) area of ground covered by the nest (A_g); and (3) volume (V). The perimeter shape of the nest opening was analyzed and varied between circular and elliptical, being considered elliptical when a clear longitudinal axis could be identified.

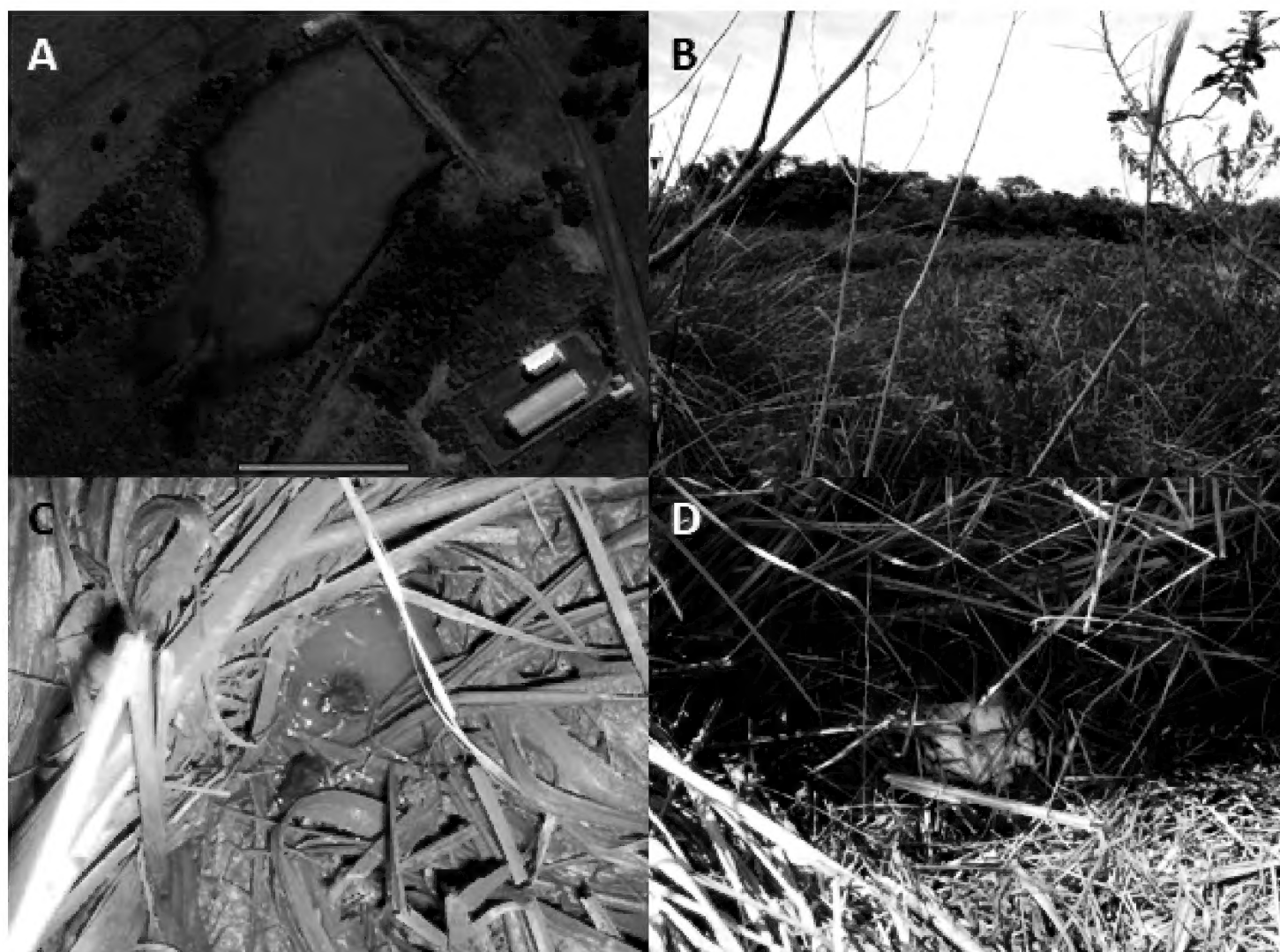


Figure 1. Overview of study site located in Ituverava, northeastern state of São Paulo, southeastern Brazil. **A)** Study area as seen by a satellite image (Map data GoogleEarth, obtained on April 23, 2013), the yellow bar is equivalent to 100 m; **B)** Typical Cerrado vegetation on the lake shore of the study area; **C)** Individuals of *Physalaemus cuvieri* before nest building; **D)** A foam nest of *Physalaemus cuvieri* in the study area.

The variables A_s and V were calculated using the following equations:

$$AS = \pi.r^2 \quad \text{Eq (1)}$$

(for circular nests), r being the radius of the circumference;

$$AS = \pi.a.b \quad \text{Eq (2)}$$

(for elliptical nests), a and b being respectively the largest and the smallest half axes of the ellipse;

$$V = \pi/6.a.b.c \quad \text{Eq (3)}$$

(for circular and elliptical nests), a and b being the diameters of the nests, perpendicular to each other, and c being the depth of the nests (Shepard and Caldwell 2005).

Humidity and temperature measurements

Measurements of relative air humidity and air temperature were taken at 2 cm around the nests (RH_o , T_o , respectively) and 2 m above (RH_2 , T_2 , respectively) using a portable thermo-hygrometer (HT-300, INSTRUTHERM, Brazil). The temperatures of three depths within the nests were also measured: on the foams surface (T_s), within the central part of the foam (T_c) and on the ground of the foam (T_g), using infrared thermometers (DT-3880 and DT-900, DELLT, Brazil). The thermal exchange between air and nests was interpreted based on data on thermal conduction and the area/volume ratio calculated by us.

Data analysis

Data were analyzed using the statistical software GraphPad Prism 5. After testing for normal distribution, the correlations between depth, area and volume of the nests were evaluated, as well as the area/volume ratios of the nests with the internal temperatures, using a two-tailed Pearson's correlation. To test for possible differences between the relative humidity around and 2 m above nests, a paired Student's t-test was performed, as well as a two-tailed Pearson's correlation. To test for possible differences between foam nest temperatures, a Repeated Measured One-Way analysis of variance (ANOVA) were used, followed by Tukey's post-hoc test. Data are given as mean \pm standard error of the mean (SEM) and a significance of $p \leq 0.05$ was considered (Zar 1984).

Results

The foam nests of *P. cuvieri* were arranged at a distance of up to two meters from the edge of the pond and with an average distance of one meter between each other. At the border of the pond, the foam nests were uniformly distributed below the vegetation, composed mainly by families Poaceae and Cyperaceae, close to the plant's body, protected from sunlight. The nests presented morphometric measurements of low variability, except for volume (Fig. 2A). Average nest depth was 11.5 ± 1.0 cm, surface area of 54.3 ± 13.2 cm² (19.6 and 157.0 cm² minimum and maximum, respectively), and a volume of 249.1 ± 26.3 cm³ (117.3 and 378.0 cm³ minimum and maximum, respectively). Area/volume ratio was 0.23 ± 0.05 cm⁻¹. The relative humidity around the foam nests ($62.8 \pm 1.4\%$) was significantly greater than two meters above ($49.5 \pm 1.7\%$; $p = 0.0127$; Fig. 2B), showing a positive significant correlation between humidity measurements ($r = 0.699$, $p = 0.0253$). All measured temperatures were significantly different from another, with the only exception being T_2 and T_o . Temperature decreased significantly from 2 cm around the nest ($T_o = 34.8 \pm 0.5$ °C), to the surface of the foam ($T_s = 28.3 \pm 0.4$ °C), the center of the foam ($T_c = 25.8 \pm 0.3$ °C), and the ground of the nest ($T_g = 24.2 \pm 0.2$ °C; Fig. 2C). Pearson's correla-

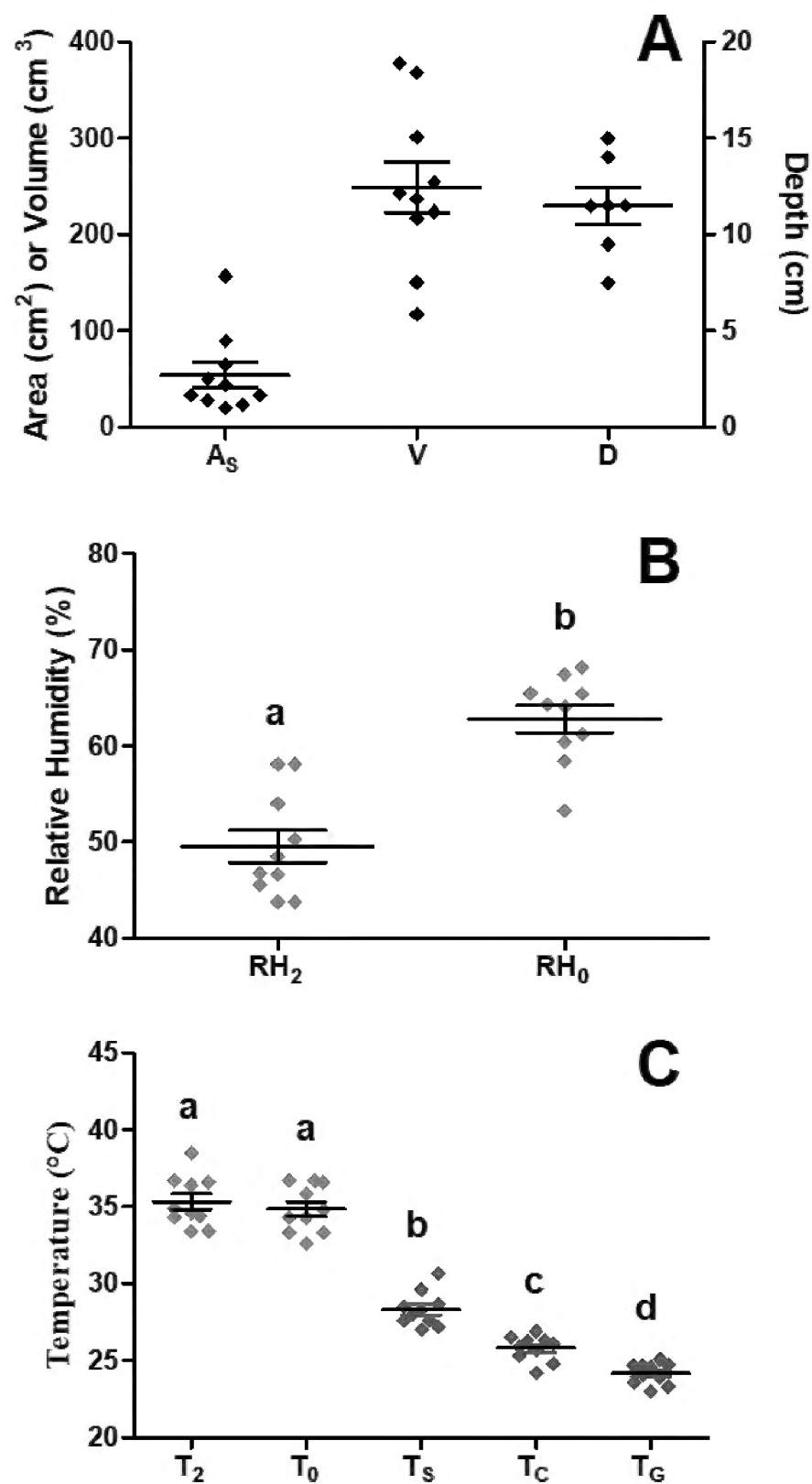


Figure 2. Morphometric and abiotic characterization of *Physalaemus cuvieri* foam nests. **A)** Foam nest measurements: depth (D), area of ground covered by the nest (A_s), nest volume (V); **B)** Measurements of relative air humidity 2 meters above the nests (RH_2) and 2 cm around the nests (RH_0); **C)** temperature measurements taken 2 m above the nests (T_2), 2 cm around the nests (T_0), on the foams surface (T_s), within the central part of the foam (T_c), and on the ground of the foam nest (T_g). Horizontal bars indicate mean and the whiskers indicate SEM. Different letters indicate significant differences between values.

tion was strongly positive ($r = 0.8897$, $p = 0.0006$) for T_2 and T_0 . T_0 was also significantly negatively correlated with D ($r = -0.8480$, $p = 0.0159$) and RH_2 ($r = -0.8097$, $p = 0.005$), while T_c was positively correlated with T_g ($r = 0.6671$, $p = 0.0351$). No significant correlation was found between morphometric and ambient variables, possibly being related to the small distance between measuring points.

Discussion

While the use of foam nest as reproductive strategy among anurans is well known in the literature (e.g. Tyler and Davies 1979; Hödl 1990, 1992; Kadadevaru and Kanamaedi 2000; Bastos et al. 2010; Cruz et al. 2016), as well as some of the foams biotechnological applications (Hissa et al. 2016; Cooper et al. 2017), there are only few studies regarding morphometry and ambient variables associated with *P. cuvieri* nests (Bokermann 1962; Barreto and Andrade 1995; Andrade 2007). Our current observations of *P. cuvieri* building nests under herbaceous vegetation close to the studied pond, corroborates findings by Barreto and Andrade (1995) and Uetanabaro et al. (2008). Andrade (2007) showed that the distribution of oviposition sites of *P. cuvieri* depends on factors such as the size and duration of bodies of water, the general climate and microclimate, the type of vegetation around these bodies of water and the presence or absence of predators. The average spatial distribution of *P. cuvieri* nests reported by Andrade (2007) was corroborated in the present study, where an average distance of one meter between two nests was observed.

The *P. cuvieri* nests showed relatively low variation in the morphometric variables analyzed, such as depth (11.5 ± 1.0 cm) and surface site (54.3 ± 13.2 cm²), except for volume (249.1 ± 26.3 cm³), as shown by the individual data points in Fig. 2A. Our data corroborate Bokermann (1962), who first studied this species in the municipality of Diadema and São José dos Campos, São Paulo State, Brazil, reporting homogeneous nest opening shapes and distribution of nests when protected by vegetation. Bokermann (1962) also carried out morphometric measurements of *P. cuvieri* nests and reported average values for the diameter of the opening varying from 7 to 9 cm, depth of 4 cm, surface area of 25.1 cm², and volume of 100.5 cm³, suggesting there is a direct relationship between the size, volume, and number of eggs. What draws attention in Bokermann's data, are the small volumes given for foam nests constructed by *P. cuvieri*, his measurements ranging from 35 to 75 cm³. Barreto and Andrade (1995), studying a population of *P. cuvieri* in the state of Maranhão, Northeastern Brazil, reported foam nest surface area of 14.8 cm², and nest volume of 57.6 cm³. Despite the differences observed for surface area and volume by Bokermann (1962) and Barreto and Andrade (1995), the area-volume ratio is very similar to our data (0.23 cm⁻¹, this study; 0.25 cm⁻¹, Bokermann (1962); 0.26 cm⁻¹, Barreto and Andrade (1995)). Kluge (1981), studying *Boana rosenbergi* (Boulenger, 1898), observed that the foam nests of this species show a great variation in their volume when comparing populations from different regions, seeming to depend on factors such as the type of substrate on which the nests are built and the vegetation around them. Bokermann (1962) did not report any information regarding the vegetation in his study site. Although the vegetation and soil reported by Barreto and Andrade (1995) were similar to our study, being composed of Poaceae and Cyperaceae, we couldn't conclude, without further information, if the *P. cuvieri* nest volumes seen in our study were influenced by the substrate. Andrade (2007) showed that the spawning size is highly dependent on the body of water near the foam nests, representing another environmental variable interfering with nest volumes, but the

water body next to our study site has not been analyzed to allow any correlation with the nest volumes obtained in the present study.

The greater relative humidity of the air around the *P. cuvieri* nests, when compared to two meters above them, could be explained by the microclimate generated by the evapotranspiration of the vegetation associated with the nests, by the humidity of the soil near the pond, as well as the lower wind velocity within the vegetation. In contrast, air temperatures around the nests did not differ from those at two meters above ground. The great incidence of sunlight in the study area throughout the day and greatly degraded shrub vegetation, providing only few shaded areas, result in high temperatures, even close to the ground. According to Murphy (2003), the temperature of the reproduction sites possesses a great influence on the behavior of adult anurans, which can choose places for oviposition with conditions more appropriate for a species' embryonic-larval development. Luza et al. (2015) have also shown that adult *Boana faber* (Wied-Neuwied, 1821) are able to select nesting sites in shallow waters with few organic particles in suspension and more covering provided by surrounding vegetation, providing developing larvae microhabitat conditions significantly different from a randomly chosen nesting site. Additionally, specifically chosen nesting sites may provide favorable microclimates that could accelerate larval development, when the nest containing eggs or larvae heats up more rapidly in sunlight than the surroundings (Dobkin and Gettinger 1985).

The air temperatures around the nests were significantly greater when compared to the surface, central and bottom temperatures of the foam nests, being some 10 °C lower in the center and at the bottom of the nest. A similar pattern has been observed for a *Leptodactylus labyrinthicus* (Spix, 1824) population from the same pond (Fernandes et al. 2016). Interestingly, Shepard and Caldwell (2005) registered temperatures inside nests 2–3 °C lower than surface temperature in much larger ($1622 \pm 667 \text{ cm}^3$) nests of *L. labyrinthicus* than the ones of *P. cuvieri* ($249.1 \pm 26.3 \text{ cm}^3$). This difference could be explained by the nest of *P. cuvieri* being some 4 cm deeper than the ones studied by Shepard and Caldwell (2005), and therefore being better isolated within the soil. Although nests of *L. labyrinthicus* can be six times more voluminous than those of *P. cuvieri*, they are built in similar fashion in the Brazilian Cerrado, with eggs being deposited into cavities on the ground along the periphery of water reservoirs (Zina and Haddad 2005). Foam nests constructed by *Pleurodema tucumanum* (Parker, 1927) (Rodríguez Muñoz et al. 2019) close to the edge of a pond and within shallow water, showed also a temperature 2–3 °C lower inside the nests, when compared to air temperature. The great temperature difference observed in the present study may be related to the fact that the nest that had their temperatures measured were well inserted into the vegetation or soil, and the temperatures measured were therefore much more influenced by soil temperatures than by air temperatures. The less variable soil temperature, together with an insulating effect from the foam, may provide stable thermal conditions for egg and larval development.

Studies on foam nests of *Engystomops pustulosus* (Cope, 1864) floating on water are somewhat contradictory regarding temperature variations. While Dobkin and Gettinger (1985) have shown temperatures inside the nests to be greater than air tem-

peratures, exceeding air temperature by 8.2 °C, Downie (1990a) did not find any significant effect of the foam on the thermal behavior of the nest when compared to the water below the nest. These differences found within the same species may be related to geographic variation, since the population studied by Dobkin and Gettinger (1985) was from an altitude of 600 m, whereas the population studied by Downie (1990a) was found at 50 m altitude. The different altitudes and resulting climate differences may contribute to the variations in nest temperatures reported for *E. pustulosus*. It is known that the temperatures of amphibian oviposition sites can be dependent on factors such as time of day, the duration of solar radiation, the presence of clouds and shadows made by vegetation onto the nests, soil characteristics and morphology of the nests (Wells 2007). Méndez-Narváez et al. (2015) have furthermore shown that for the leptodactylids *Physalaemus fischeri* (Boulenger, 1890), *Leptodactylus fuscus* (Schneider, 1799), and *Leptodactylus knudseni* (Heyer, 1972) foam nests reduce temperature fluctuations within the nests when compared with surrounding temperatures, and that foam nests placed onto water do protect the nest less from temperature fluctuations than more terrestrial nests. The nests made by *P. cuvieri* showed a low area/volume ratio ($0.23 \pm 0.05 \text{ cm}^{-1}$) when compared to a nest not placed into a depression in the ground, which means that, on average, nests showed a volume five times greater than their opening area, resulting in a very small area for heat exchange between the foam and the surrounding air. This observation corroborates data from Cardoso (1981), who also showed that *P. cuvieri* nests have a low area/volume ratio. Cardoso (1981), unconcerned about studying thermal relationships, suggested that such a low area/volume ratio acts as a protective factor against predation of eggs and larvae, especially if the vegetation around the nest is degraded. The vegetation in our study area showed a great degree of degradation, due to anthropogenic influence onto the area around the pond. Ryan (1985) and Zina (2006) also observed a low area/volume ratio for foam nests of other species, and proposed that this factor could provide greater protection against the desiccation of *Engystomops* and *Pleurodema* eggs.

The low area/volume ratio seems, however, also to be important for thermal relationships, as a low nest surface exposed to air would reduce heat exchange between *P. cuvieri* nests and air. The lower the area/volume ratio, the smaller the surface and center temperatures would be and vice versa. Another factor that could contribute to the observed reduction in nest temperatures is the vertical orientation of the *P. cuvieri* nests, which increase the distance between the eggs/larvae and the outside air. The low thermal conductivity of the air (Halliday et al. 2012), trapped in the form of small bubbles, which supplies oxygen for the development of embryos and larvae (Seymour and Loveridge 1994; Evans et al. 1996; Einum et al. 2002), also functions as thermal insulation. Another important observation would be the participation of the part of the nest making contact with the ground, which could act as a heat sink. Some water accumulates at the bottom of the nest, and due to the greater specific heat of water (Halliday et al. 2012), heat is being transferred from the inside of the nest into the ground.

Building foam nests as reproductive strategy has evolved independently several times among anurans (Faivovich et al. 2012), and biophysical properties of the differ-

ent types of foam are currently being investigated (Cooper et al. 2017). The foam contains mostly surfactant proteins and carbohydrates, and the varying stability of different kinds of foam are probably related to different types of these proteins and carbohydrates being present in varying proportions among different taxa (Cooper et al. 2017). No data are currently available regarding the chemical composition of *P. cuvieri* foam.

Conclusion

We conclude, that (1) the foam nests of *P. cuvieri* are exposed to a wetter microclimate when compared to the air above the vegetation, (2) the foam nests of *P. cuvieri* showed internal temperatures well below atmospheric values in the period during which the measurements were taken, and (3) the morphometry and foam of *P. cuvieri* nests seem to influence the way in which they perform thermal exchanges with their surroundings, maintaining a stable temperature suitable for the development of eggs, embryos, and larvae. However, more detailed studies are needed in order to understand how nest temperatures might fluctuate over a 24-hour periods or over an entire incubation period, and to establish the thermal sensitivity of *P. cuvieri* eggs and tadpoles from different populations to such temperature variations.

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